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(71) Applicant *(for all designated States except US)*: IMPERIAL COLLEGE OF SCIENCE TECHNOLOGY AND MEDICINE [GB/GB]; Sherfield Building, Exhibition Road, London SW7 2AZ (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: HERNANDEZ, Diana [GB/GB]; Neurogenetics Unit, Imperial College School of Medicine, Norfolk Place, London W2 1PG (GB). MEE, Joseph [GB/GB]; National Institute for Medical Research, The Ridgeway, London NW7 1AA (GB). MARTIN, Joanne [GB/GB]; The Royal London Hospital, Dept. of Histopathology, London E1 1BB (GB). TYBULEWICZ, Victor [GB/GB]; National Institute for Medical Research, The Ridgeway, London NW7 1AA (GB). FISHER, Elizabeth [GB/GB]; Neurogenetics Unit, Imperial College School of Medicine, Norfolk Place, London W2 1PG (GB).

(74) Agents: MASCHIO, Antonio et al.; D. Young & Co., 21 New Fetter Lane, London EC4A 1DA (GB).

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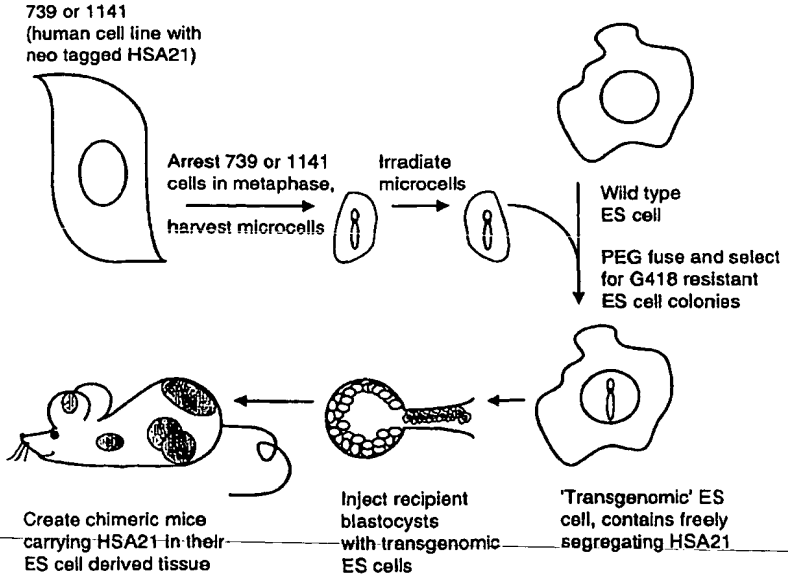
(54) Title: CHROMOSOME TRANSFER (XMMCT) TO ES CELLS, INDUCED DURING THE EXPOSURE OF MICROCELLS TO RADIATION

(57) Abstract

The present invention discloses a method for producing a transgenomic ES cell comprising the whole or a part of a heterologous chromosome. The method comprises the steps of: a) tagging a chromosome or a part of a chromosome in a cell with a dominant selectable marker; b) inducing microcell formation in the cell; c) isolating the microcells and irradiating them; d) fusing the microcells to ES cells; e) selecting for hybrids comprising the chromosome or part of the chromosome.

Irradiation microcell mediated chromosome transfer (XMMCT) protocol, as described in 'Methods' for placing HSA21 sequences in mice.



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CHROMOSOME TRANSFER (XMMCT) TO ES CELLS, INDUCED DURING THE EXPOSURE OF MICROCELLS TO RADIATION

The present invention relates *inter alia* to a transgenic animal model for diseases which involve complete or partial aneuploidy, such as Down Syndrome. Moreover, the invention relates to a method for transferring large DNA molecules, such as chromosomal fragments, large genes or complete chromosomes, into cells.

At least 8% of all human conceptions have major chromosome abnormalities and the frequency of chromosomal syndromes in newborns is 0.5%. Despite these disorders making a large contribution to human morbidity and mortality, we have little understanding of their aetiology and little molecular data on the importance of gene dosage to mammalian cells. Trisomy 21, which results in Down syndrome (DS), is the most frequent aneuploidy in humans (1 in 600 live births world-wide) and is the most common single cause of mental retardation. To investigate the molecular genetics of DS we create strains of mice that carry all or part of HSA21 as a freely segregating extra chromosome. In order to produce these transgenomic animals we transfer HSA21 (whole or partial) into mouse embryonic stem cells (ES) using irradiation microcell mediated chromosome transfer (XMMCT).

This technology is applicable to many other areas of research, including the transfer large chromosomal regions into ES or other cells, and the investigation of other aneuploidy syndromes.

The invention therefore provides, in a first embodiment, a method for producing a transgenomic ES cell comprising the whole or a part of a heterologous chromosome, the method comprising the steps of:

a) tagging a chromosome or a part of a chromosome in a cell with a dominant selectable marker;

b) inducing microcell formation in the cell;

c) isolating the microcells and irradiating them:

d) fusing the microcells to ES cells;

e) selecting for hybrids comprising the chromosome or part of the chromosome.

The selection of the chromosome of fragment thereof which it is desired to transfer will depend on which disease it is desired to study. For example, in the study of Down Syndrome, an aneuploidy involving trisomy of chromosome 21 in humans, human chromosome 21 is transferred. The chromosome may be transferred to cells derived from its natural source, or from a heterologous organism. "Heterologous", as used herein, merely refers to the fact that the natural state has not been maintained; thus, an extra copy of an otherwise endogenous chromosome is a "heterologous" chromosome.

Preferably, the tagging of the chromosome is carried out by insertion of a marker gene, preferably a *neo* cassette, into a non-essential region of the chromosome, in the vicinity of the gene locus or loci which it is desired to transfer. This may be achieved by any suitable means, but particularly preferred is targeted homologous recombination, techniques for which are known in the art. Particularly preferred embodiments are described below.

The chromosomal fragments transferred by the method of the present invention are large chromosomal fragments, not normally transferable by conventional transformation techniques. They may be present in the recipient ES cell as independent "extra" chromosomes, or as fragments integrated within the chromosomes resident in the cell.

The invention, moreover, provides ES cells comprising heterologous chromosomal fragments or chromosomes when produced by the above method.

Further embodiments of the invention relate to transgenic animals incorporating ES cells as described above. The production of transgenic animals, using ES cells and otherwise, is well known in the art, and described for example in Manipulating the Mouse Embryo, 2nd Ed., by B. Hogan, R. Beddington, F. Costantini, and E. Lacy. Cold Spring Harbor Laboratory Press, 1994; Transgenic Animal Technology, edited by C. Pinkert. Academic Press, Inc., 1994; Gene Targeting: A Practical Approach, edited by A. L. Joyner. Oxford University Press, 1995; Strategies in Transgenic Animal Science, edited by G. M.

Monastersky and J. M. Robl. ASM Press, 1995; and Mouse Genetics: Concepts and Applications, by Lee M. Silver, Oxford University Press, 1995.

Briefly, The embryonic stem (ES) cells that are used for targeted insertion are derived from the inner cell masses of blastocysts (early mouse embryos). These cells are pluripotent, meaning they can develop into any type of tissue. ES cells must be maintained on a layer of feeder cells, typically mouse embryo fibroblasts that have been irradiated to prevent them from dividing. ES cells must be passaged every 2-3 days to keep them from differentiating (and losing pluripotency).

Pluripotent ES cells can be reintroduced into a blastocyst to generate a transgenic animal. Donor females are mated, blastocysts are harvested, and 10-15 ES cells are injected into each blastocyst, which is then implanted into the uterus of a pseudopregnant recipient. By choosing the appropriate donor strain, the detection of chimeric offspring (i.e., those in which some fraction of tissue is derived from the transgenic ES cells) can be as simple as observing hair and/or eye color. If the transgenic ES cells do not contribute to the germline (sperm or eggs), the transgene cannot be passed on to offspring.

First generation animals are necessarily chimeric, that is do not contain an inserted chromosomal fragment in every cell. Only the tissues of the animal derived from the transfected ES cells comprise the extra DNA element. However, by crossing transgenic animal chimeras, pure transgenic lines may be created. Alternatively, selective crossing may be used to generate transgenic lines in which the presence of the heterologous chromosomal element may be tailored, to vary the dosage of the element within the organism as a whole, or to direct the element to specific tissues within the organism.

Moreover, since the method of the invention permits the generation of ES lines comprising different chromosomal elements, generated by independent recombination events after the microcell irradiation procedure, the effect of mutations in the transferred locus or loci may be studied. Crossing of transgenic lines may be used to develop novel lines having different characteristics and containing different sections of DNA.

Further embodiments of the invention, which are intended to illustrate rather than limit the scope thereof, will be apparent from the following example. Where literature references given are incomplete, the correct reference may be identified easily and in a straightforward manner by inspecting the relevant volume of *Index Medicus* or any other suitable bibliographic publication. Reference is made also to the following figures:

Fig. 1 Homologous recombination near the locus D21S55 with the pTS55SN targeting construct. pTS55SN contains the neo and TK genes for double selection, and has homologously recombined into HSA21 at a site 15.5kb from D21S55. Correctly targeted HT1080 cell lines thus contain a 5.1 kb HindIII fragment rather than the wild type 8.1kb HindIII fragment, when hybridised with our flanking probe as shown. Restriction enzyme sites are as indicated: B is Bam HI, G is Bgl II, H is HindIII and RI is EcoR I.

Fig. 2 Scheme of irradiation microcell mediated chromosome transfer, as described in 'Methods'.

Fig. 3 The transgenomic cell lines and their HSA21 content, as shown by a range of HSA21 specific markers, including the loci indicated. Cell line names are shown above the black bars that indicate HSA21 regions; cell line are followed by irradiation levels (rads) in brackets.

Fig. 4 Irradiation microcell mediated chromosome transfer (XMMCT) protocol, as described in 'Methods' for placing HSA21 sequences in mice.

In slightly more detail, Fig. 1 is a scheme of homologous recombination near the locus *D21S55* with the pTS55SN targeting construct. pTS55SN contains the *neo* and *TK* genes to allow positive and negative selection[41a] and has 9.3kb of isogenic DNA from a site 15.5kb from *D21S55*. Correctly targeted HT1080 cell lines were identified by the presence of an 8.2kb *Hin*d III fragment rather than the wild type 5.1kb *Hin*d III fragment, when hybridized with the 1.2kb flanking probe (data not shown).

In slightly more detail, Fig. 3 FISH (human Cot1 DNA probe hybridized to metaphase spreads of transchromosomal ES cell line) and IRS FISH (Alu PCR products from each transchromosomal cell line as probe, hybridized to normal human male metaphase spreads) of transchromosomal cell lines containing HSA21. *a*, FISH of transchromosomal cell line 43-Q; *b* IRS FISH with probe derived from transchromosomal cell line 43-Q; *c*, FISH of transchromosomal cell line 44-2;

d IRS FISH with probe derived from transchromosomal cell line 44-2; *e*, FISH of transchromosomal cell line 47-14; *f* IRS FISH with probe derived from transchromosomal cell line 47-14.

Example 1:

Creating an ES cell line containing large regions, or the whole of, human chromosome 21.

To create transgenomic ES cell lines, a human donor cell line containing HSA21 tagged with a dominant selectable marker is required. A neomycin resistance gene (neo) is inserted into the locus *D21S55* by homologous recombination within the human fibrosarcoma cell line HT1080 (Rasheed, 1974; Fig 1). For this experiment a genomic library is constructed and two constructs are prepared therefrom for electroporation into HT1080 cells. Out of 2569 cell lines that are selected in G418 and gancyclovir, two different lines, 739 and 1141, have a correctly targeted neo gene at *D21S55*.

The *D21S55* locus is chosen as being within a region central to the genes involved in the major aspects of DS, particularly mental retardation. The HT1080 cell line has previously been used as a donor in microcell experiments (Saxon, 1985).

Having targeted *D21S55* we proceed to transfer regions of HSA21 into mouse ES cell lines by fusion with irradiated human microcells.

Briefly, the donor cell lines 739 and 1141 are treated with colcemid to induce microcell formation (see Methods). The microcells are isolated using a percoll gradient in the presence of cytochalasin B (Stubblefield, 1992) and then irradiated to kill any remaining donor human cells and produce breaks in the human chromosomes (Dowdy, 1990; Koi, 1993). A series of experiments is carried out with irradiation levels ranging from 3,500 to 50,000 rads. Irradiated microcells are fused to D3 ES cells using polyethylene glycol, and the resulting transgenomic lines are then selected in G418 for those containing either the whole, or fragments of, human chromosome 21, including the targeted locus near *D21S55* (Fig 2).

By this method we isolate 53 different ES cell lines with growth characteristics and morphologies identical to the parental D3 line (see Fig. 3). These 53 transgenomic cell lines are characterised with respect to their human and mouse DNA content by: (i) karyotyping all cell lines to determine average chromosome number; (ii) FISH analysis with Cot1 human DNA to determine human chromosome number and whether human DNA had integrated or is freely segregating; (iii) PCR and Southern blot analysis with 20 well mapped markers spaced approximately 2 Mb apart on HSA21q, to determine which portions of the chromosome are present in the transgenomic cell lines. HSA21 has an excellent physical map, and therefore the physical distances between these markers are well defined (Ichikawa, 1993).

The karyotype and FISH analysis show that the transgenomic cell lines fall into three classes. The majority has one freely segregating human chromosome that is maintained in the absence of selection after at least 6 passages on a normal D3 ES cell karyotype. A second group has two or more freely segregating human chromosomes and the third group (2 cell lines) has a region of human DNA that has integrated into the mouse genome.

Southern blot and PCR analysis with our set of HSA21 markers on the transgenomic cell lines with one freely segregating human chromosome shows that this chromosome appears to be either full length HSA21 or has different portions of HSA21 extending distal and proximal from D21S55, attached to a 21 centromere. The presence of the

HSA21 centromere is determined by the specific restriction enzyme pattern of the probe D21Z1 which distinguishes this centromere from others, especially the closely related sequence on HSA13. It is known from radiation hybrid experiments that centromeres tend to be maintained after irradiation of donor chromosomes, thus our cell lines are giving the expected results [Goodfellow, 1990]. It has been suggested that fragments containing centromeres may have a selective advantage, for unknown reasons [Walter, 1993]. In most of the cell lines contiguous stretches of DNA appear to have been maintained as determined by our panel of HSA21 markers. While more detailed restriction mapping studies are required to confirm this, we note that two other similar studies, one of which carried out detailed pulse field electrophoresis analysis found almost no rearrangements of human chromosome fragments after irradiation [Koi, 1993; Siden, 1992].

We do not find any obvious correlation between irradiation level and the physical length of HSA21 that is present in the transgenomic ES cell lines. In radiation hybrid experiments with X rays, Cox and colleagues irradiated the Chinese hamster-human somatic cell hybrid, CHG3, which contains a single copy of HSA21. They found the number of human fragments per radiation hybrid cell followed a Poisson distribution, with an average of 5 HSA21 fragments per cell at 8000 rads [Goss, 1975; Cox, 1990]. Siden and colleagues used gamma irradiation in similar experiments with a cell hybrid containing human chromosomes 3 and X on a Chinese hamster background and found that while human chromosome content is dependent on irradiation dose, there is variability between individual recipient cell lines [Siden, 1992; Goodfellow, Trends in Genetics, 1993].

The development of this technology is an important step for creating mouse models of human aneuploidy syndromes. Currently such models fall into three classes, and all have disadvantages when attempting to both detect candidate genes and model aspects of the human phenotype. In the first class lie the mouse chromosomal aneuploidies, which are very rare and almost always lethal unless they are partial trisomies. Partial trisomies can be helpful for phenotypic studies but currently have limited use in dissecting out dosage sensitive candidate genes or regions in human chromosomes.

Genetic manipulation by cre-lox recombination for example [Ramirez-Solis, 1995], could increase the range of partial trisomies available, however there is not an exact correlation between human and mouse chromosomes and thus a mouse partial trisomy may carry three copies genes that have homologues on two or more human chromosomes. The second class of mouse, the YAC transgenic mouse, results in a more tractable model for human aneuploidy syndromes. For example in the case of DS, YACs from HSA21 have been transferred into mice [Smith, 1995]. By assaying for a reproducible cognitive deficit, and then creating new transgenics with decreasing stretches HSA21 DNA, a locus has been mapped that is likely involved in neurological aspects of DS. However this method of assaying for DS genes is too laborious for scanning a whole chromosome or chromosome arm, and has the disadvantage that very large genes will not fit on most YACs and even small genes may be interrupted. Thirdly, various single gene transgenic mice have been proposed as models for aspects of DS, but these involve just one gene (of the ~1000 thought to lie on HSA21), usually with inappropriate levels and patterns of expression.

These three types of aneuploidy model provide supportive data for our transgenomic ES cells and their use to make mice. From the partial trisomy 16 mice it is clear that extra chromosomes can be stably inherited in mice and, at least in the female line, the mice are fertile [Davisson, 1993; Reeves, 1995; Epstein, 1997]. The YAC and single gene transgenics indicate that though no predictions can be made for specific genes, most human transgenes are expressed from their own promoters and function normally in a mouse environment.

Transgenomic mice and cell lines provide us with flexibility of the mouse as a genetic system for assessing the effects of gene dosage. For example we can place the extra chromosome onto different genetic backgrounds, to assess the effect on phenotype (which may be helpful in DS, which has a very variable phenotype); or we can undertake further rounds of genetic manipulation within transgenomic ES cell lines, to test candidate genes for dosage effects, by reducing a 3 dose back to a 2 dose in the same cell line, by gene targeting. Our method is also applicable to investigations other

than those concerned with gene dosage. One example of the use of transgenomic approach is to transfer the complete human immunoglobulin region into a mouse.

Example 2:

Creating transgenic mouse lines from transgenomic ES cells comprising the human HSA21 locus.

An ES line containing the entire human HSA21 locus as a single independently segregating human chromosome is selected for generation of trasngenic mice. Blastocysts are harvested from donor animals, and 10-15 ES cells are injected into each blastocyst. The blastocysts are then implanted into the uterus of pseudopregnant recipients and allowed to develop to term.

Mice generated by this procedure are tested using the karyotyping, FISH and PCR approaches described in Example 1. The mice are shown to be chimeric, incorporating the human chromosome derived from the ES cell line used in some but not all tissues.

In detail, 120 blastocysts (derived from C57/B6 mice) are injected with ES cells from the line 49-1, which contains a small portion of human chromosome 21 (5Mb). The blastocysts are transferred into 11 pseudopregnant fosters (mouse strain (CBAXB10)F1), and allowed to go to term.

8 of the foster mice give birth to live litters comprising a total of 12 mice. Of the 12 mice 9 are clearly chimeric as judged by coat colour, with the degree of chimerism ranging from 5-60%.

Of the nine chimeric pups born, 4 are females and 5 are males. One of the males dies at weaning (3 weeks).

We confirm the presence of human sequences in the tissues of the chimeric mice by extracting DNA from a tail biopsy, and using the DNA to amplify sequences from the human specific marker D21855 by PCR.

We observe that the mice have abnormal gait, the severity of which increases with the degree of chimerism. The mouse with the highest degree of chimerism has also abnormal posture resembling kyphosis.

5

Methods

Targeting HT1080 cells.

A genomic library from the human fibrosarcoma cell line, HT1080[Rasheed, 1974] is
10 constructed, from which two isogenic constructs are built, pPNT.S55 and pTS55SN, for electroporation into HT1080 cells (Fig 1). Both constructs are based on the pPNT targeting vector[Tybulewicz, 1991]. pPNT.S55 contains the D21S55 locus with the pNT PGK-neo and PGK-TK genes. pTS55SN contains genomic DNA 15.5kb away from D21S55 with an SV40-neo construct and the same pNT PGK-TK gene. HT1080
15 cells are electroporated with the linearised constructs as described in [Itzhaki, 1997; Itzhaki, 1991]. Cells are then plated at limiting dilutions in 96 well plates and double selection is applied 24 hours after electroporation (400mg/ml G418 and 1mM ganciclovir). 10 days post electroporation colonies are picked and expanded into 24 well plates. We have determined that confluent HT1080 cells from one well of a 24
20 well plate are sufficient for half to be frozen down and half to be used for DNA isolation for Southern blotting. Restricted HT1080 DNA is hybridised with a flanking probe that detects a size difference from wild type if homologous integration has occurred (Fig 1). A total of 71x106 cells are electroporated with the first construct pPNT.S55, 1360 clones are recovered and analysed by Southern blotting but no
25 homologous recombinants are isolated. Using the second construct, pTS55SN, a total of 68x106 cells are electroporated and 1209 clones are isolated. Clone DNA is analysed by digestion with HindIII and probed with a 1.2kb flanking fragment that detects a 5.1kb band in HT1080 DNA and an 8.2kb band in homologous recombinants (Fig 1). We isolate 2 cell lines, 739 and 1141, that have been correctly targeted. Further
30 restriction enzyme analysis and hybridisation results indicate that both cell lines contain a single integrant. It is unknown if a gene has been interrupted by the construct, but

from published restriction maps it is believed that the neo gene has integrated into intron 1 of the GIRK1 gene .

Production, harvesting and irradiation of microcells.

739 or 1141 targeted HT1080 cells are induced to form microcells by prolonged arrest in colcemid (0.04mg/ml colcemid for 48 hours. Cells are then harvested by trypsinisation and loaded into centrifuge tubes containing percoll and cytochalasin B and centrifuged (12,500 rpm for 1 hour) to separate microcells from cell debris and whole cells. Following centrifugation the microcells are isolated from the top of the gradients.

The isolated microcells are then irradiated using a Nordion Irradiator with a Co source at a rate of 1000 rads/min.

Fusion of microcells to mouse ES cells.

Irradiated microcells are counted and mixed in a tube with equal numbers of ES cells that had been previously harvested using conventional techniques. The cell mix is pelleted and resuspended in 10mg/ml PHA-P (Difco) and microcells are allowed to agglutinate for 30 min at 37°C. Following agglutination cells are fused in suspension using PEG 1500 (Boehringer Mannheim). The PEG suspension is diluted and cells are allowed to recover for 30 min at room temperature. Following incubation cells are plated at low densities onto feeder layers in standard ES cells media and cultured overnight. The next day the medium is replaced with medium containing G418 at 500mg/ml. Colonies are picked 2 weeks later.

Genotyping transgenomic ES cell lines

Transgenomic cell lines are analysed by (i) chromosome karyotyping: conventional techniques are used to produce metaphase spreads; (ii) FISH: Labelling and hybridisation of Cot1 human DNA to metaphase spreads is carried out essentially as

described in [Siden, 1992; Lichter, 1990]. Briefly chromosome slides are treated with RNase for 1 hour at 37°C and dehydrated in an ethanol series. The slides are then denatured in formamide at 75°C for 3 min, and immediately dehydrated in a cold ethanol series and hybridised to a similarly denatured biotin labelled total human DNA probe, under coverslips in a humid chamber at 37°C overnight. After hybridisation slides are washed in 50% formamide at 42°C for 20 min, followed by two 2xSSC washes at 42°C for 10 min. Hybridised human DNA is detected by a sandwich technique using FITC-avidin/antiavidin antibodies/FITC-avidin treatment in series. After staining in propidium iodide antifade solution, the slides are analysed using a fluorescent microscope; (iii) DNA marker analysis: PCR and Southern blotting/probe hybridisation are carried out using standard techniques.

Production of Chimeric Mice

Chimeric animals are produced as described above, according to the procedures of Bradley, "Production and Analysis of Chimaeric Mice", in *Teratocarcinomas and embryonic stem cells. A practical approach"*, E.J. Robertson (Ed.), IRL Press, Oxford, UK (1987), which is incorporated herein by reference.

More details on this aspect of the present invention are now mentioned below.

As indicated above, at least 8% of all human conceptions have major chromosome abnormalities and the frequency of chromosomal syndromes in newborns is >0.5%[1a]. Despite these disorders making a large contribution to human morbidity and mortality, we have little understanding of their aetiology and little molecular data on the importance of gene dosage to mammalian cells. Trisomy 21, which results in Down syndrome (DS), is the most frequent aneuploidy in humans (1 in 600 live births, up to 1 in 150 of all pregnancies world-wide[2a, 3a]) and is the most common known genetic cause of mental retardation[4a]. To investigate the molecular genetics of DS we are creating mice that carry all or part of human chromosome 21 (HSA21) as a freely segregating extra chromosome. To produce strains of these 'transchromosomal'

animals we have placed a selectable marker into HSA21 and transferred the chromosome (whole or partial) from a human somatic cell line into mouse embryonic stem (ES) cells using irradiation microcell mediated chromosome transfer (XMMCT); chimeric mice have been created from the 'transchromosomal' ES cells. Chimeras from one transchromosomal ES cell line show consistent cranio-facial and skeletal abnormalities; this experiments delineates a region of chromosome 21 that appears to contain a dosage sensitive gene which gives rise to developmental abnormalities when present in three copies. This novel experimental strategy is applicable to investigations requiring the transfer of large chromosomal regions into ES or other cells, and, particularly, the modelling of other human aneuploidy syndromes.

To create transchromosomal ES cell lines, we needed a human donor line containing HSA21 tagged with a dominant selectable marker. We created two new cell lines, 739 and 1141, by inserting a neomycin resistance gene (*neo*) near the locus *D21S55* by homologous recombination in the human cell line HT1080[5a] (Fig. 1). The *D21S55* locus was chosen for targeting because it lies within a region thought to contain gene(s) involved in the major aspects of DS, particularly mental retardation[6a, 7a]. The HT1080 cell line was chosen because it is one of very few human transformed cell lines known to be a successful donor in microcell mediated chromosome transfers[8a]. Both 739 and 1141 were used in our subsequent experiments.

Having targeted *D21S55* we proceeded to transfer regions of HSA21 into mouse ES cells by irradiation microcell mediated chromosome transfer (XMMCT[9a, 10a]) (Fig. 2). Briefly, the donor cell lines 739 and 1141, were treated with colcemid to induce microcell formation[11a] and then centrifuged in a percoll gradient containing cytochalasin B[12a, 13a]. The microcell layer was collected and irradiated to kill any remaining donor human cells and to produce breaks in the human chromosomes[14a]; a series of experiments was carried out at irradiation levels of 3.5, 10, 20, 30, 40 and 50 krads. Irradiated microcells were fused to D3 ES cells using polyethylene glycol and then colonies were selected in G418 (Fig. 2). Resistant colonies were picked and from then on, cultured in non-selective medium.

We carried out 14 XMMCT experiments, in which a total of $4.2x10^9$ microcells were fused to $1.4x10^9$ ES cells. From these we isolated 49 different ES cell lines that were shown by Southern blot analysis to contain the *neo* gene, HSA21 sequences adjacent to its site of integration and the *D21S55* locus. These lines have growth characteristics and morphologies identical to the parental D3 cells and are derived from experiments carried out at all 6 irradiation levels. FISH analysis with human Cot1 DNA as the probe was undertaken to determine human chromosome number and whether the donor human DNA had integrated or was freely segregating in the lines; it is known from radiation fusion experiments that centromeres tend to be maintained in cell hybrids after irradiation of donor human chromosomes[15a-17a]. We found three categories of transchromosomal cell line: 27 lines have one freely segregating human chromosome; 20 cell lines have two or more freely segregating human chromosomes; 2 cell lines contain a fragment of human DNA that has integrated into the mouse genome (Fig. 3 *a*, *c*, *e* and data not shown). Human chromosomes are maintained in the absence of selection after at least 6 passages.

We undertook IRS-FISH analysis to determine the chromosomal origin of the human DNA in our 27 transchromosomal cell lines that contain one freely segregating human chromosome (Fig. 3 *b*, *d*, *f*). Labelled Alu PCR products from 21 of the lines hybridize to HSA21 only in human male metaphase spreads; products from 6 of the 27 lines hybridize to HSA21 and to HSA 10, 14 , 16p, 16p, 18 and 19 respectively, indicating that material in addition to HSA21 was transferred into the ES cells. These 6 cell lines were derived from XMMCT at 3.5, 30, 40 and 50 krads.

DNAs from the 21 HSA21 transchromosomal cell lines were subjected to PCR and Southern blot analysis with 32 markers spaced on average 1 - 2 Mb apart on HSA21, to ascertain the integrity and content of the human chromosome. In these studies, HSA21 content of the 21 transchromosomal cell lines containing one freely segregating human chromosome derived exclusively from HSA21. *a,* PCR and Southern blot analysis with

a representative set of HSA21 markers were analysed. Cell line name, marker name and

expected size of fragment were indicated in the final figure. Transchromosomal cell lines were analysed with D3 wild type ES cell line, RA21 (a cell hybrid containing HSA21 only on a mouse background), 1141(H) (targeted human cell line described above), as controls. *D21S11* (GDB: 188664), *D21S82* (GDB: 192002), *IFNAR* (GDB: 185155), *D21S49* (GDB: 196282), primer details were as given in GDB. *MNB*: whole human cDNA hybridized to a *Hind* III digest of genomic DNAs. *D21Z1*: genomic DNA fragment (GDB: 166570) hybridized to an *Eco*R I digest of genomic DNAs. ***b,*** Cell line names and microcell irradiation dose (in parentheses) were shown as the vertical bars that indicate the HSA21 regions thought to be present, as judged by the panel of HSA21 markers shown on the left. HSA21 loci have been positioned approximately according to the relative distances on the LDB map[42a]. *D21S5* is a short arm marker, *D21Z1* detects the HSA21 centromere, and loci *D21S16* through to *COL6A1* run in order from the centromere to 21q telomere. According to the LDB map the length HSA21 from 21pter to 21qter is 50Mb and the length of 21q from the centromere to 21qter is 49 Mb. All marker details were given in GDB, except for *D21S55* which is described in[43a], *SOD1* described in[44a] and *SIM2*, *MNB*, *GIRK2* and *ETS2*. Note that chimeric mice have been produced from cell lines 49-1 and 46-1.

In particular, we found that HSA21 appears to be either full length or has different stretches extending distal and proximal from *D21S55*, attached to a human centromere. This centromere is most likely derived from HSA21 as judged by our results with the probe *D21Z1*. The stretches of HSA21 appear likely to be contiguous, and while more detailed restriction mapping is required to confirm this, we note that other studies find almost no rearrangements of human chromosome fragments after irradiation[10a, 16a] and no evidence of radiation breakage 'hotspots' on HSA21[18a].

From our sur sample size from each irradiation level we see a trend that those cell lines produced from lower irradiation doses tend to have larger human chromosomes,

whereas those from higher doses tend to have smaller human fragments. A study of a large set of radiation hybrids found that in general donor human chromosome fragment size was dependent on irradiation dose, but there was great variability between individual cell lines produced at the same dose[16a].

The 21 transchromosomal lines containing one freely segregating human chromosome derived exclusively from HSA21 were karyotyped to determine mouse chromosome number; 15 cell lines had a euploid modal number of 40 mouse chromosomes plus 1 transchromosomal HSA21 (>10 metaphases examined), in the remaining 6 lines >50% of cells were aneuploid with respect to mouse chromosome number. We have found similar results in other experiments with gene-targeted D3 cell lines and thus the aneuploid lines are most likely derived from the normal D3 background, rather than being an artefact arising from the XMMCT protocol.

We injected transchromosomal cell lines into C57BL/6 blastocysts to create chimeric mice carrying HSA21 chromosome portions. One of our first cell lines for injection is 49-1, which we have shown contains a human genomic region extending from at least *D21S394* to *ETS2*. In these studies, HSA21 content and human gene expression of transchromosomal cell line 49-1 and chimeric mice made with this line. *a*, PCR analysis of 9 markers that define the extent of the transchromosomal region around *D21S55* in genomic DNA from cell line 49-1 and tail biopsy DNA of 8 chimeric mice derived from injections of this line into C57BL/6 blastocysts (49-1.1F, .2M, .3M, .4F, .5F, .6F, .7M, .8M; M - male; F - female). Also included was the PCR analysis of genomic DNA from the parental D3 ES cell line, the donor targeted human cell line 1141(H), a monochromosomal cell hybrid containing HSA21 as its only human component on a mouse background (RA21), and tail biopsy DNA from a C57BL/6 mouse. Expected fragment sizes of PCR products were shown. *AML1* (GDB: 185177); *SIM2* (SIM2F: AAAGCCAACAAACCAAGAC, SIM2R: TTGTAGCAAACACGAGCC); *D21S336* (GDB: 190709); *MNB* (MNBF: GTTGTAAAGGCATATGATCGTGTG, MNBR: GTTCATGAGCTCAAGAAGTCGCAC); *D21S55* ([43]); *GIRK2* (GIRK2F:

CCCAAAATACTACACATCC, GIRK2R: GTTTGTCTTCAGCTCACC); *ETS2* (GDB: SHGC-6939); *HMG14* (GDB: 184390); *D21S515* (GDB: 191991); *D21S49* (GDB: 196282). Three chimeras (49-1.1F, .3M, .4F) showed no amplification of the markers, possibly because of low levels of chimerism (<10%). *b,* a diagram of HSA21 content of cell line 49-1 was also prepared. Distances between markers are taken from the LDB map[42a] except for *SIM2* to *GIRK2* which comes from [45a]; 220kb is the distance between *D21S55* and the 5' end of *GIRK2*. *c,* RT-PCR results of four genes that map within the 49-1 HSA21 region (*SIM2*, *MNB*, *GIRK2* and *ETS2*). The expected sizes of RT-PCR products were shown; in each case the primers span a large intron (>20kb) which precludes amplification of genomic DNA under these conditions. In addition RT-PCR analysis of *GdX*, a ubiquitously expressed X-linked mouse gene, was included to control for the presence of RNA in every mouse sample. The *GdX* primers span a small intron (115bp); the expected sizes of RT-PCR (126bp) and genomic DNA (241bp) products were shown[30a]: *SIM2* (SIM2RNAF: GATGACCGCTGTCCTCACGGC, SIM2RNAR: CATATACTGCCTGATCTTCAAG), *MNB* (MNBRNAF CAACCTCTAACTAACCAGAGGCG, MNBRNAR TCCACACGATCATATGCCTTTAC), *GIRK2* (GIRK2RNAF: TTCATCCCGTTGAACCAGACGG, GIRK2RNAR CCCATCCTCCAGGGTCAGGAC) and *ETS2* (ETS2RNAF TACTCAGCTCTGAGCAGGAGTTTC, ETS2RNAR AACGTTCGATGTCATCCAGTGTTA), *GdX* (GdXRNAF: GGCAGCTGATCTCCAAAGTCCTGG, GdXRNAR: AACGTTCGATGTCATCCAGTGTTA).

In particular, so far we have recovered 28 live pups from injections with 49-1; 15 of these pups are chimeric as judged by coat color. The process of chromosome transfer into ES cells could alter their ability to chimerize with adult mouse tissues. However, PCR analysis of tail biopsy DNA shows the chimeras contain the expected 49-1 HSA21 derived sequences, so the XMMCT protocol does not appear to compromise differentiation of ES cells. Furthermore these results also indicate the human

chromosome fragment is maintained *in vivo*. It is possible that the XMMCT protocol could abolish human gene expression within the ES cells. To investigate this we carried out RT-PCR analysis of four genes known to map within the 49-1 human region: *SIM2*, *MNB*, *GIRK2* and *ETS2*. RNA was made from adult brain from chimeric mouse 49-1.8M, human adult brain (frontal neo cortex), and various controls including the 49-1 cell line. The RT-PCR primers are human specific, and exonic but each pair spans one intron, thus ensuring we detect transcribed products only. All four genes were expressed in the brain of the 49-1.8M chimera. *GIRK2* which does not appear to be detectable by RT-PCR until E14 of development[19a] was not expressed in the cell line 49-1, suggesting that appropriate gene regulation is maintained in the transchromosomal cell lines.

The 49-1—C57BL/6 chimeric mice clearly looked morphologically different from their non-chimeric littermates. We investigated these differences by carrying out a standard phenotype analysis, the primary screen of the SHIRPA protocol[20a], on 4 male chimeras (16 weeks old) compared to 4 age-matched C57BL/6 males. The mice were scored for a range of morphological and behavioural features; all C57BL/6 mice had test scores within the normal range except one with no demonstrable startle response, an occasional finding suggesting a hearing defect in this strain. In comparison, all four chimeras had shortened necks and their heads were shorter than controls; this appears to be due to a relative facial shortening seen particularly in the eye to nose length and the interocular distance, with a small degree of antero-posterior skull shortening. Control mice had a mean body weight of 30.2g (range 29-32g), that for the transchromosomal chimeras was 34g (range 23-42g). The highest level chimera (estimated by coat colour as ~60% agouti), 49-1.8M, had a markedly reduced weight of 23g. Additional phenotypic abnormalities seen in 49-1.8M included a hunched posture, relative endophthalmos of the left globe compared to the right, shortening of the forelimbs, bilateral ulnar deviation of the forepaws and left wrist (radiocarpal) subluxation. Visual placing in this animal was poor, but this is likely due to forelimb dysfunction rather than a visual defect since additional study using the finger approach (where the head turns towards an approaching digit) was normal on both sides. Fore and hind limb tone in 49-1.8M was

reduced, as was grip strength; on the wire manoeuvre the mouse had difficulty pulling up the hindlimbs, but this may relate to poor grasp with forelimbs rather than a truncal or hindlimb weakness. 49-1.7M also had difficulty with this test; the two other chimeras had no abnormalities on functional testing.

X ray analysis was carried out on 49-1.8M, 4 C57BL/6 males and 2 chimeras consisting of the same C57BL/6 (blastocyst) and D3 (ES cell) combination in which the D3 cells were heterozygous for a *Brca2* gene targeting mutation[21a]. In these studies, Dorso-ventral radiograph of chimera 49-1.8M at five months old, taken post-mortem following formalin fixation, using a Faxitron X-ray machine at 30Kev for 1 min was taken. Film used was Kodak standard. Chimera 49-1.8M showed abnormal curvature of the spine (kyphosis), and shortened forelimbs.

In particular, in comparison to the controls 49-1.8M showed a shortening of the cervical region and severe kyphosis affecting primarily the upper thoracic spine; no specific bony vertebral abnormalities were noted to account for the deformity. There was shortening and valgus deformity of the forelimbs, chiefly affecting the distal portion of the limb with further lateral (ulnar) deviation of the forepaw, including the carpus and digits. Similar skull and forelimb shortening has since been observed in more recently produced chimeras (with comparable percentage chimerism) derived from ES cell line 49-1. The skull was short with anteroposterior shortening of the facial bones, but no significant broadening was seen. No bony abnormalities of the orbit were found. Internal examination of 49-1.8M showed gastric dilation with food retention, however, no atresia or structural stenosis was seen in the small intestine. Other histological examination of all major organs, including brain, showed no significant abnormalities of macroscopically normal organs in all test and control chimeras.

In contrast to the phenotypic abnormalities observed in the 49-1 chimeric mice, 10 chimeras made with cell line 46-1 showed no obvious morphological or behavioural differences compared to C57BL/6 or 129/Sv controls (stage 1 SHIRPA protocol). We hypothesize that the skeletal abnormalities seen in the 49-1 chimeras are due to the

effects of a dosage sensitive gene (or genes) present in this cell line but not the 46-1 cell line. One of the differences between these two transchromosomal ES cell lines is the presence of human *ETS2* in 49-1 but not in 46-1 (Fig. 4). We note that transgenic studies have shown that a subtle increase in *Ets2* expression gives rise to mice with a variety of skeletal abnormalities including shortened snouts, abnormally shaped heads, shorter necks and kyphosis, and similar skeletal abnormalities are found in trisomy 16 mice (which have 3 copies of *Ets2*)[22a, 23a]; also human DS individuals tend to have shorter necks and shorter long bones than non-DS individuals[24a]. Thus the abnormalities seen in our 49-1 chimeric mice may be due to the presence of the additional human *ETS2* gene.

The development of this technology is an important step for creating mouse models of human aneuploidy syndromes. Currently such models fall into three classes, each with limitations: in the first class lie the mouse aneuploidies. Although only partial autosomal aneuploidies survive beyond birth, these can be helpful for phenotypic studies but have limited use for the fine mapping and isolation of dosage sensitive genes. Nonetheless from the partial trisomy 16 mice that carry 3 copies of HSA21 gene homologs, it is clear that extra chromosomes can be stably inherited in mice[25a-27a]. Genetic manipulation by Cre-induced recombination[28a], could increase the range of partial aneuploidies; however each human chromosome has homologous linkage groups on a number of mouse chromosomes and thus it will be difficult to create mouse trisomies with gene sets that are identical to human trisomies. YAC transgenic mice provide a more tractable model for human aneuploidy syndromes. In the case of DS, HSA21 YAC transgenic mice have been assayed for a reproducible cognitive deficit, and then by creating new transgenics with decreasing stretches of HSA21 DNA, a locus has been mapped that is likely involved in neurological aspects of DS[29a, 30a]. This locus appears to be the Minibrain, *MNB*, gene, which is present in three copies in the 49-1 cell line. However this method of assaying for dosage sensitive genes is too laborious for scanning a whole chromosome or chromosome arm, and has the disadvantage that large genes (such as the HSA21 gene *DSCAM*[31a]) will not fit onto YAC transgenic constructs and genes with distant regulatory sequences may be

interrupted. Lastly, various single gene transgenic mice have been proposed as models for aspects of DS, for example, but these involve just one gene (of the ~800-1000 thought to lie on HSA21), usually with inappropriate levels and patterns of expression[32a]. Nevertheless the YAC and single gene transgenics indicate that most human transgenes are expressed from their own promoters and can give rise to abnormal phenotypes from a subtle increase in gene product dose[23a, 30a].

Our research shows that XMMCT is a successful approach for placing HSA21 into mice and thus potentially modelling DS. In addition we make one more important point for XMMCT modelling of human aneuploidy syndromes *per se* (rather than just creating transchromosomal mice): it is essential to use a human donor cell line, as we have done, and not a human-mouse cell hybrid as in the studies of Tomizuka *et al.*, because there is no straightforward method to determine if donor mouse DNA has integrated into the recipient ES cell -- from our results integration occurs in 22% of chromosome transfers. We used a human somatic cell as a donor, in which we had targeted a dominant selectable marker into HSA21; in future it may be possible to tailor the DT40 chicken cell system[34a] to XMMCT studies of human aneuploidy, by capitalizing on its high rate of homologous recombination, ability to form microcells and the genomic differences between the human/chicken donor and mouse recipient.

Transchromosomal mice and cell lines provide us with the flexibility of a model genetic system for assessing the molecular consequences of aneuploidy. For example, for particular traits, dosage sensitive candidate genes could be mapped and then their effects assayed by further rounds of genetic manipulation to reduce a 3 dose back to a 2 dose in mice. We can also start to address an essential feature of DS, the difference in penetrance and severity of most traits, by placing the extra chromosome onto different genetic backgrounds and determining which regions of the genome affect these traits. This is relevant to the non-DS population as many of the characteristic features of DS also occur in other individuals, not apparently trisomic for HSA21.

Methods

Targeting HT1080 cells.

We initiated a two step chromosome walk by screening a genomic phage library from the human fibrosarcoma cell line, HT1080[5a] with a 2.5kb *Eco*R I fragment from the *D21S55* locus. Thus we isolated approximately 22kb of *D21S55* flanking DNA from which we built an isogenic construct, pTS55SN, for electroporation into HT1080 cells. pTS55SN contains 9.3kb of genomic DNA derived from a site 15.5kb away from *D21S55* and is based on the pPNT targeting vector[35a]. An SV40-neo cassette was inserted into a *Hin*d III site in the middle of the 9.3kb homology region and a PGK-TK cassette was placed adjacent to this genomic DNA (Fig. 1). The construct was linearized by digestion at the unique *Not* I site of the pPNT vector. A total of $68x10^6$ HT1080 cells were electroporated with the linearized construct as described in[36a, 37a]. Cells were then plated at limiting dilutions in 96 well plates and double selection was applied 24 hours later (400mg/ml G418, 5μM ganciclovir). A total of 1209 colonies survived and these were picked 10 days after electroporation and expanded into 24 well plates. DNA from the double selected colonies was analysed by digestion with *Hin*d III and probed with a 1.2kb flanking fragment that detects a 5.1kb fragment in parental HT1080 DNA and an 8.2kb fragment in homologous recombinants (Fig. 1). From the 1209 colony DNAs we found 2 cell lines, 739 and 1141, with a correct targeting event. Further restriction enzyme analysis and hybridization results indicated that both cell lines contain a single integrant and the structure of the targeted locus was as expected (data not shown).

Production, harvesting and irradiation of microcells.

739 or 1141 targeted HT1080 cells were induced to form microcells by prolonged arrest in colcemid (0.04mg/ml colcemid for 48 hours). 24, 175cm flasks of cells were harvested by trypsinization, resuspended in a mixture of serum-free DMEM and percoll (1:1) and loaded into 50ml polycarbonate Oak Ridge centrifuge tubes (Sorval)

containing cytochalasin B (10mg/ml) and centrifuged (16,000xg) for 1 hour to separate microcells from cell debris and whole cells. Following centrifugation the microcells were recovered from the top of the gradients, washed and resuspended in serum free DMEM. Microcells were filtered though 8mM then 5mM polycarbonate filters (Costar) to enrich for small microcells containing single chromosomes. The filtered microcells were irradiated at room temperature using a Nordion g Cell Irradiator with a ^{137}Cs source at a rate of 1000 rads/min.

Fusion of microcells to mouse ES cells.

Irradiated microcells were counted using a hemocytometer and mixed with equal numbers of ES cells that had been previously harvested using conventional techniques (typical numbers ranged from 100-300x10^6 microcells and 100x10^6 ES cells). The cell mix was pelleted and resuspended in 10ml 10mg/ml PHA-P (Difco) and microcells were allowed to agglutinate for 30 min at 37°C. Following agglutination cells were fused in suspension using 2ml PEG 1500 (50%, Boehringer Mannheim). The PEG suspension was diluted with 20ml serum free DMEM and cells were allowed to recover for 30 min at room temperature. Following incubation, cells were plated at a density of 2x10^6 cells/ 10cm dish onto feeder layers in standard ES cell medium and cultured overnight. The next day the medium was replaced with ES cell medium containing G418 (500mg/ml) and cells were selected in this for 2 weeks. Surviving colonies were then picked and all further culture took place in medium without selection.

Genotyping transchromosomal ES cell lines.

Karyotyping: conventional techniques were used to produce metaphase spreads from the transchromosomal cell lines and chromosomes were counted after Leishman's staining. FISH analysis: Labelling and hybridization of human Cot1 DNA to transchromosomal metaphase spreads was carried out essentially as described in[16a, 38a]. Briefly, chromosome slides were treated with RNase for 1 hour at 37°C and dehydrated in an ethanol series. The slides were then denatured in formamide at 75°C for 3 min, and

immediately dehydrated in a cold ethanol series and hybridized to a similarly denatured biotin labelled human Cot I DNA probe, under coverslips in a humid chamber at 37°C overnight. After hybridization slides were washed in 50% formamide at 42°C for 20 min, followed by two 2xSSC washes at 42°C for 10 min. Hybridized human DNA was detected by a sandwich technique using FITC-avidin/antiavidin antibodies/FITC-avidin treatment in series. After staining with propidium iodide/DAPI antifade solution, the slides were analysed using a Leitz Aristoplan fluorescent microscope. IRS-FISH analysis: This protocol was carried out essentially as in[39a] and the TC65 Alu-PCR primer was used to generate the probe[40a]. DNA marker analysis: PCR and Southern blotting/probe hybridization were carried out using standard techniques.

Genotyping transchromosomal chimeric mice.

DNA marker analysis: PCR and Southern blotting/probe hybridization were carried out using standard techniques. DNA was prepared from tail biopsies of 8 chimeric mice.

RT-PCR of transchromosomal chimeric mouse tissue.

RNA was prepared from brain of control mice, chimeric mouse 49-1.8M, human adult brain (frontal neo cortex), D3 and 49-1 ES cell lines, using the Qiagen Rneasy total RNA kit. RNA was then reverse transcribed using the Stratagene RT-PCR kit and first strand cDNA was used as template for PCR.

Phenotypic and histological studies.

Phenotype testing was performed according to the first stage of the SHIRPA protocol[20a] (see http://www.mgu.har.mrc.ac.uk/handbook/Mproto.html). Tissue for histological analysis was immersion fixed *in situ* in formol saline. Samples for light microscopy were processed to paraffin wax using standard protocols and sections were

cut at 2 mm. Sections were dewaxed in xylene, rehydrated through graded alcohols and stained with haematoxylin and eosin.

Modifications will be appearent to those skilled in the art.

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Claims

1. A method for producing a transgenomic ES cell comprising the whole or a part of a heterologous chromosome, the method comprising the steps of:

a) tagging a chromosome or a part of a chromosome in a cell with a dominant selectable marker;

b) inducing microcell formation in the cell;

c) isolating the microcells and irradiating them:

d) fusing the microcells to ES cells;

e) selecting for hybrids comprising the chromosome or part of the chromosome.

2. A method according to claim 1, wherein the cell contains the heterologous chromosome in an autonomously replicating form.

3. A method according to claim 1 or claim 2, wherein the cell contains more than one heterologous chromosome or part thereof.

4. A method according to any preceding claim, wherein the chromosome, or part thereof, is derivable from human chromosome 21 (HSA 21).

5. A method according to any preceding claim wherein the chromosome or part thereof is tagged by inserting a marker gene, which is preferably a *neo* gene, thereon.

6. A method according to claim 4 wherein HSA 21 is tagged by inserting a *neo* cassette in the vicinity of the D21S55 locus.

7. A method according to any preceding claim wherein irradiation of the microcells is carried out at a does of between 3500 and 50,000 rads, preferably at a rate of 1000 rads/min.

8. A transgenomic ES cell when produced by or obtainable by the method of any one of the preceding claims.

9. A transgenic organism when generated from or obtainable by generation from a transgenomic ES cell according to claim 8.

10. A transgenic organism according to claim 9 which is a mouse.

11. A transgenic mouse model for Down Syndrome, the mouse comprising cells derived from a transgenomic ES cell line which contains the whole or a part of HSA 21 in the form of an autonomously replicating chromosome.

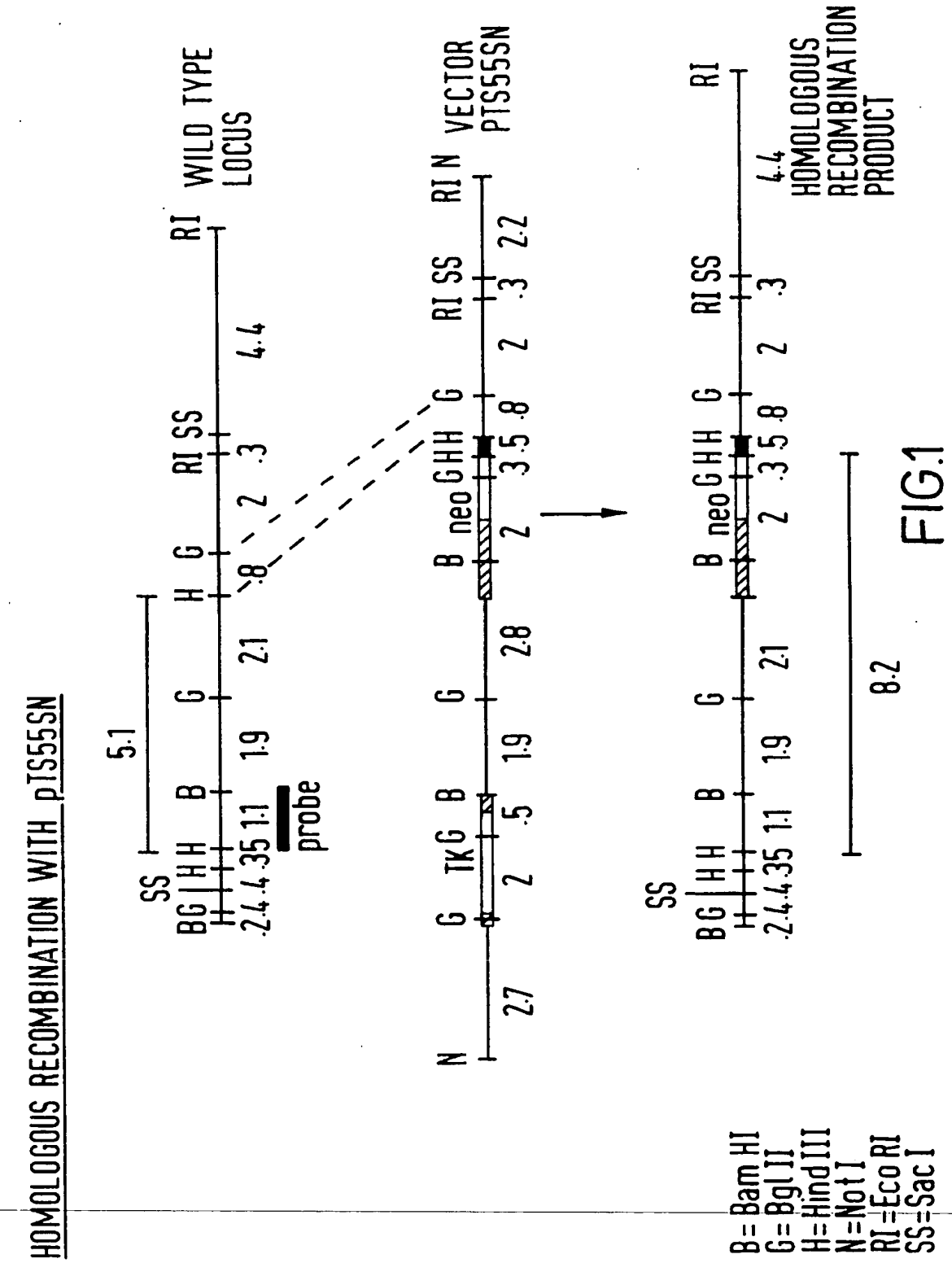
HOMOLOGOUS RECOMBINATION WITH pTS55SN
WILD TYPE LOCUS
VECTOR PTS55SN
HOMOLOGOUS RECOMBINATION PRODUCT
probe
B= Bam HI
G= Bgl II
H= Hind III
N= Not I
RI=Eco RI
SS=Sac I

FIG.1

XMMCT

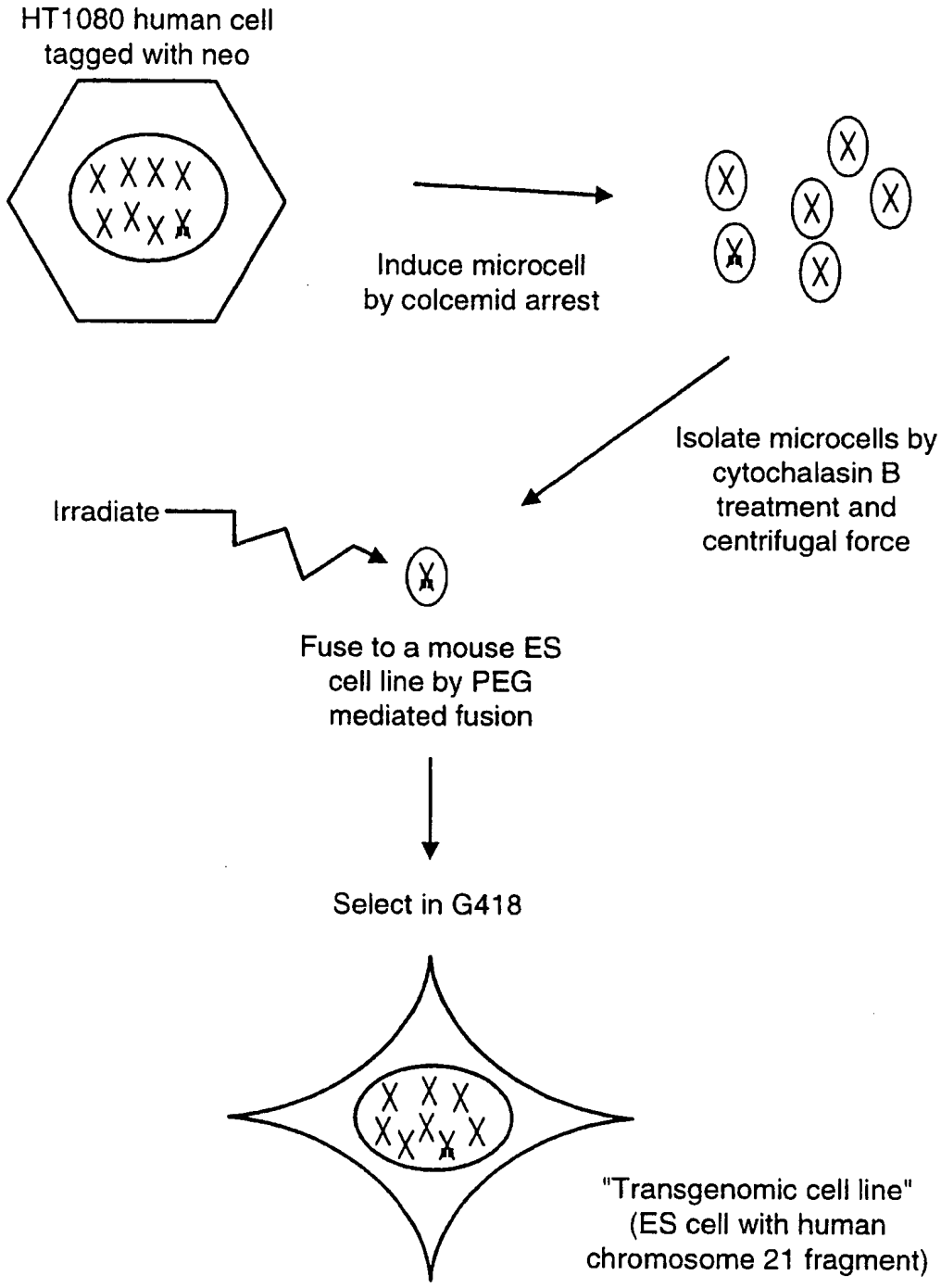
HT1080 human cell tagged with neo
Induce microcell by colcemid arrest
Isolate microcells by cytochalasin B treatment and centrifugal force
Irradiate
Fuse to a mouse ES cell line by PEG mediated fusion
Select in G418
"Transgenomic cell line" (ES cell with human chromosome 21 fragment)

FIG.2

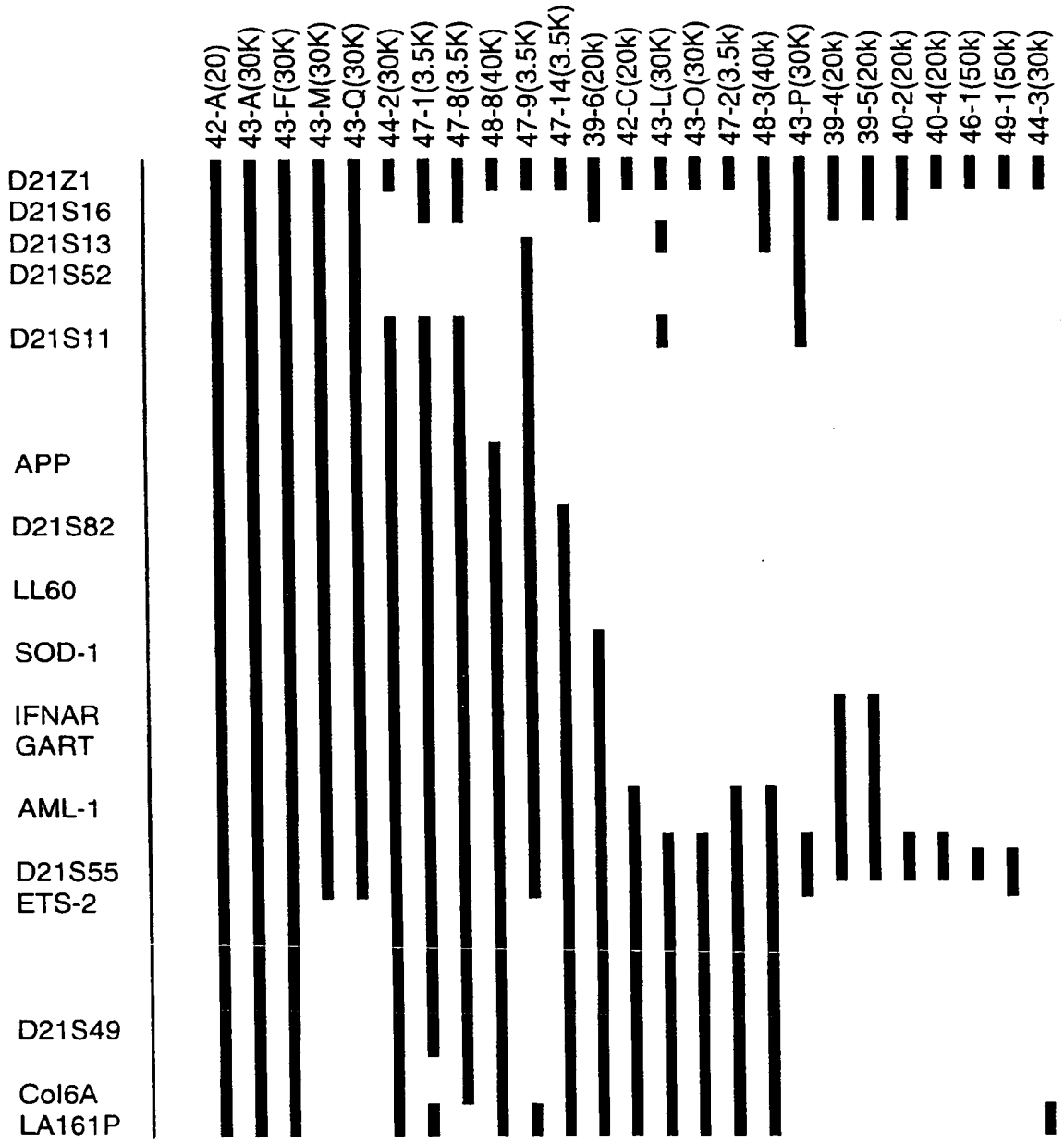
HYBRIDS
42-A(20)
43-A(30K)
43-F(30K)
43-M(30K)
43-Q(30K)
44-2(30K)
47-1(3.5K)
47-8(3.5K)
48-8(40K)
47-9(3.5K)
47-14(3.5K)
39-6(20k)
42-C(20k)
43-L(30K)
43-O(30K)
47-2(3.5k)
48-3(40k)
43-P(30K)
39-4(20k)
39-5(20k)
40-2(20k)
40-4(20k)
46-1(50k)
49-1(50k)
44-3(30k)
D21Z1
D21S16
D21S13
D21S52
D21S11
APP
D21S82
LL60
SOD-1
IFNAR
GART
AML-1
D21S55
ETS-2
D21S49
Col6A
LA161P

FIG.3

Irradiation microcell mediated chromosome transfer (XMMCT) protocol, as described in 'Methods' for placing HSA21 sequences in mice.

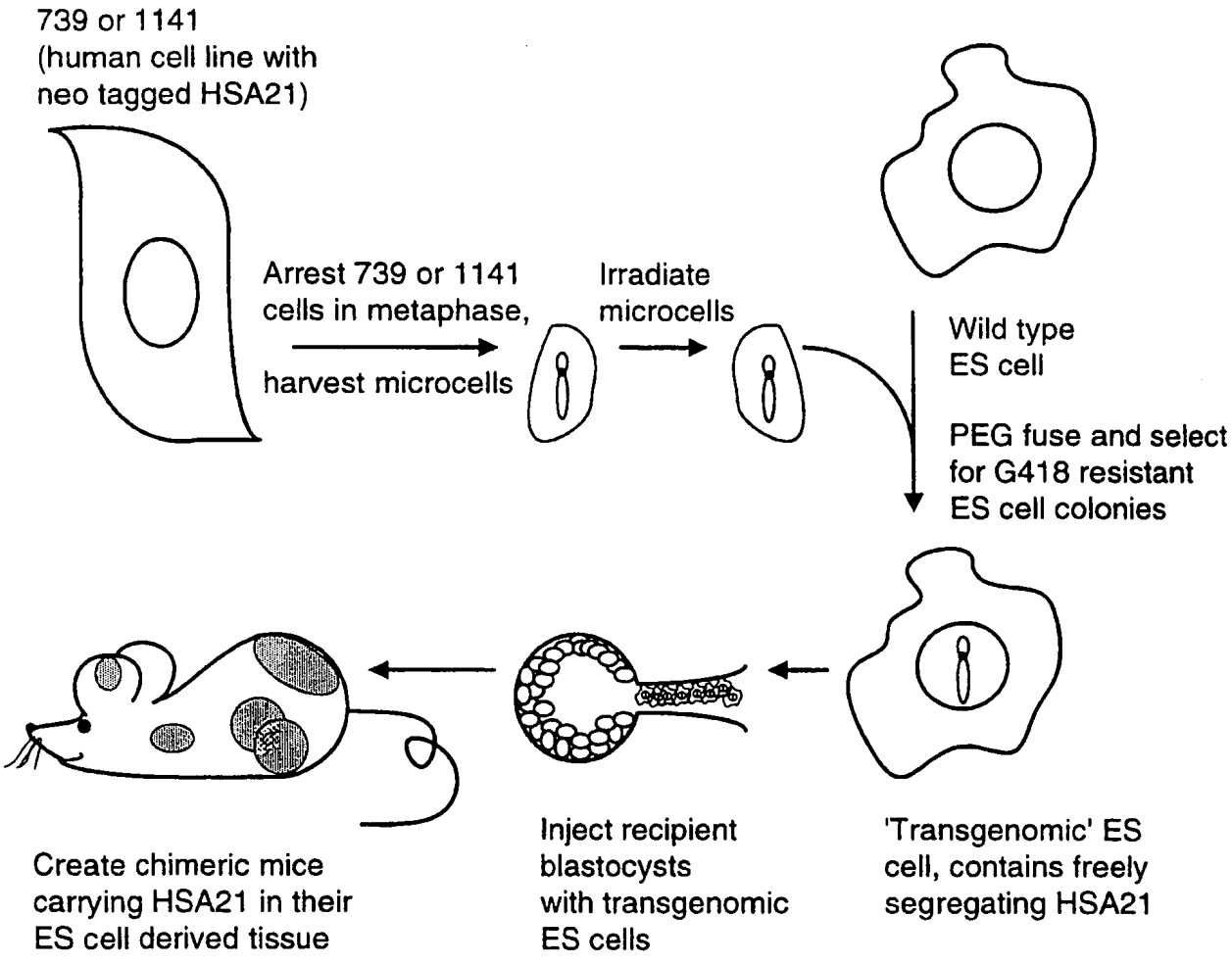

FIG.4

INTERNATIONAL SEARCH REPORT

	National Application No
	PCT/GB 98/01054

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/00 A01K67/027 C12N5/06 C12N5/10

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,O	HERNANDEZ, D. ET AL.: "Modelling aspects of Down syndrome in mice" INTERNATIONAL CONFERENCE ON CHROMOSOME 21 AND MEDICAL RESEARCH ON DOWN SYNDROME, BARCELONA SPAIN, 14 - 15 March 1997, XP002075875	1-6,8-11
P,X	& CYTOGENETICS AND CELL GENETICS, vol. 77, no. suppl 1, June 1997, page 29 see abstract	1-6,8-11

X	WO 97 07671 A (ISHIDA ISAO ;KIRIN BREWERY (JP); OSHIMURA MITSUO (JP); TOMIZUKA KA) 6 March 1997	1-3,5, 7-10
P,X	-& EP 0 773 288 A 14 May 1997 see the whole document	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 September 1998	10/09/1998

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/01054

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	KOI, M. ET AL.: "Tumor cell growth arrest caused by subchromosomal transferable DNA fragmens from chromosome 11" SCIENCE., vol. 260, no. 5106, 16 April 1993, pages 361-364, XP002075876 LANCASTER, PA US cited in the application see figure 1 -----	7

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 98/01054

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9707671 A	06-03-1997	AU 6837696 A	19-03-1997
		EP 0773288 A	14-05-1997
		EP 0843961 A	27-05-1998